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Challenges of collecting blow from small cetaceans

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Abstract. We trialed the collection of blow samples using a waterproof electric multirotor (quadcopter) drone from two free-ranging dolphin species, the abundant and approachable bottlenose dolphin (*Tursiops aduncus*) and the less common and boat shy humpback dolphin (*Sousa sahulensis*). This drone was fast, maneuverable, and quiet compared to other drones commonly used in studies of cetaceans and relative to their hearing thresholds. We were successful in collecting blow samples from four individual dolphins (three bottlenose dolphins and one humpback dolphin) in two groups. The success of obtaining samples was dependent on the individual dolphin's activity. We were successful in sampling when dolphins were resting and socializing but found that socializing dolphins were not predictable in their surfacing and direction and therefore do not recommend drone sampling socializing dolphins. The suitability and preference of the sampling technique over biopsy sampling is highly dependent on the dolphin activity. We also attempted to extract DNA from the blow samples with the aim of assessing the feasibility of using blow sampling by drone for population genetic studies. We were unsuccessful in extracting DNA and recommend that others attempting to sample dolphin blow with a drone should prioritize collecting a larger volume of blow that may yield adequate concentrations of DNA to be amplified. Blow sample volume could potentially be increased by sampling with more absorbent materials.

Key words: animal welfare; behavior; biopsy; DNA; remotely piloted aircraft; sampling; unmanned aerial vehicle.

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INTRODUCTION

Wild marine mammals can be notoriously difficult to study as they spend most of their time underwater and out of sight of the observer. Consequently, this can present challenges when attempting to collect important ecological data from free-ranging animals. Remotely piloted aircraft (e.g., drones) are increasingly being used as the platform of choice to monitor wildlife in many different circumstances (Linchant et al. 2015, Gonzalez et al. 2016) and have enabled the collection of data that were previously difficult if not impossible to collect. In studies of marine

mammals, drones have been used with thermal imaging to assess populations (Seymour et al. 2017), determine body condition and identify individual whales (Dawson et al. 2017, Christiansen et al. 2018), count dugongs (Hodgson et al. 2013) and pinnipeds (McIntosh et al. 2018), acquire photographic images to estimate population size (Colefax et al. 2018, Hodgson et al. 2018), record behavior (Goebel et al. 2015, Fiori et al. 2017, Torres et al. 2018), and collect blow samples from whales (Pirotta et al. 2017, Domínguez-Sánchez et al. 2018). The collection of biological samples is of particular interest, as drones are potentially a noninvasive tool with minimal impact on the animals (Christiansen et al. 2016).

Blow samples collected from cetaceans can be analyzed for DNA to investigate population structure (Frère et al. 2010), analyzed for hormones for stress (Hunt et al. 2014, Thompson et al. 2014) and health (Apprill et al. 2017), and screened for epizootics, microbiota (Pirotta et al. 2017), and viruses (Geoghegan et al. 2018). Such studies have been successfully executed on large cetaceans such as baleen whales (Domínguez-Sánchez et al. 2018, Harcourt et al. 2019) as these animals have proven the most suitable to approach and sample with this emerging technology. While blow samples collected from dolphins can be suitable for DNA analysis, samples collected so far have been from captive or habituated dolphins that could be approached closely (Frère et al. 2010). Sampling blow from free-ranging dolphins that cannot be easily and consistently approached closely remains challenging.

In the last two decades, multiple studies of population structure of dolphins have relied on the collection of tissue samples using a PAXARMS (modified rifle with free-floating biopsy dart; Krützen et al. 2002), crossbow (Patenaude and White 1995), stranded or bycaught animals (Bilgmann et al. 2011), or a biopsy pole for bow-riding dolphins (Bilgmann et al. 2006). These methods are poor or unworkable for species that tend to be difficult to approach closely by boat such as tropical dolphins, which are often more elusive (Parra et al. 2002) than other more approachable species such as bottlenose dolphins (*Tursiops* sp.; Wilson et al. 1999). One such evasive species was the focus of this study, the Australian humpback dolphin (*Sousa sahulensis*; hereafter referred to as humpback dolphin). While this species has been successfully sampled using a PAXARMS in Australia, it has taken many years of intensive effort to acquire sufficient sample sizes for population analyses (Brown et al. 2014, Parra et al. 2018). Accordingly, a method where the species could be approached closer but with minimal effect on the dolphin would be preferable.

This study trialed the feasibility of using a drone to collect blow samples from free-ranging dolphins, targeting both bottlenose (*Tursiops aduncus*; more abundant and easier to approach) and humpback dolphins, and then attempted to extract DNA from the blow samples.

Methods

Sampling equipment

In May 2018, one week was devoted to locating both dolphin species by boat in the Dampier Archipelago, Western Australia, with the aim of using a drone to collect blow samples from them. We used a custom-built waterproof 4-motor electric multirotor (quadcopter), which was fast, maneuverable, and relatively quiet compared to other drones used for cetacean research. Given drone noise is generally a function of disk loading (aircraft mass/swept propeller area), our rationale was that our custom-built drone produced less noise because the disk loading (6.47 kg/m^2) was significantly less and it was smaller and lighter than other off-the-shelf drones used to investigate cetacean hearing thresholds and behavioral response, that is, DJI Inspire (9.88 kg/m^2) and Swellpro SplashDrone (7.88 kg/m^2; Christiansen et al. 2016, Fettermann et al. 2019). A sterile petri dish was mounted on the top and at the front of the drone with a hinged mechanism that could be opened and closed remotely. The drone was operated in manual mode (with no GPS or auto-leveling assistance) with a live video feed by a pilot with extensive experience flying at low altitude over water and in close proximity to marine mammals. Another video camera (GoPro Hero 4 Session, GoPro, San Mateo, California, USA) was used to record and review the sampling attempts and confirm the identity of the individual targeted. For comprehensive details on the drone design, see Pirotta et al. (2017).

Sampling method

Once a group of dolphins was detected, their activity was observed before making a decision as to whether it was suitable to approach with the drone for a sampling attempt. Activity was assigned to one of four mutually exclusive types foraging, traveling, socializing, or resting (Karniski et al. 2015). The decision about whether to sample depended on whether dolphins spent enough time at the surface. The drone pilot would attempt to follow the dolphins at low altitude (<5 m) and swoop to 0.5–1 m above an individual dolphin at the time of, or immediately following, an exhalation. Once a sample was collected, the drone returned to the research vessel and the sample from the petri dish was collected using the following protocol. The petri dish was (in order) (1) dry-swabbed with a Rowe Scientific cotton buccal swab and (2) rinsed with TE buffer pH 8.0 to suspend the blow sample, which was then swabbed multiple times (2–3) until the petri dish appeared dry. These swabs were then labeled and stored on dry ice for several days until they could be processed at the laboratory.

DNA extraction and amplification

Each swab sample (above) was individually used for DNA extraction with the inclusion of a human mouth swab sample as an extraction positive control. All DNA extractions were performed using the Qiagen QIAamp DNA Investigator Kit designed specifically for forensic samples, including swabs, and following the manufacturer's instructions for cotton or Dacron swabs. It should be noted that this kit and protocol differs from Frère et al. (2010). DNA was eluted in 50 μL of ATE buffer, quantified using the Qubit dsDNA Broad-Range Assay Kit on a Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and visualized via gel electrophoresis by running 10 μL of DNA extract on 1.5% agarose gels stained with GelRed (Fisher Biotech, Wembley, Western Australia, Australia). To confirm the presence of dolphin DNA in blow samples, an approximately 600-bp fragment of the mitochondrial control region (D-Loop) was amplified by polymerase chain reaction (PCR) using previously published primers H00034 (Rosel et al. 1994) and D-Loop (Cunha et al. 2005). Polymerase chain reactions were carried out in 25 μL reaction containing 1× PCR buffer, 2 μmol/L dNTPs, variable $MgCl_2$, and 1 U Taq polymerase (Invitrogen, Carlsbad, California, USA). Thermal cycling conditions were initial denaturation at 95°C for 3 min, followed by 35 or 40 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min; and a final extension step of 72°C for 7 min. We performed PCRs using variable reaction conditions, including adjusting $MgCl_2$ concentration (2.5, 3.0, 4.0 mmol/L), adjusting DNA volume (4, 6, 10 μL), annealing temperature (52–60°C), and the number of PCR cycles (35, 40). In all PCRs, we included both a positive (DNA from *Grampus griseus* tissue sample that previously amplified for the D-Loop primers) and a negative control. PCRs were visualized by gel electrophoresis as described above.

Results

We undertook 40 drone flights (ranging from <1 min to a maximum of 12 min) over seven different groups of dolphins (2 bottlenose and 5 humpback; Table 1). The maximum total flight duration of the drone over a single group was 42.1 min, the average was 23.1 min. On average, five attempts (close approach <1 m swoop with dish open) were made per dolphin group, but a maximum of 16 attempts was made on one bottlenose dolphin group. Note that there was a maximum of five attempts made per flight. We were successful in obtaining samples from four individual dolphins, from two different groups, including three from bottlenose dolphins and one from a humpback dolphin. Video footage of a successful sampling attempt can be found in Video S1. For the majority of drone flights (21 of 40), dolphins were engaged in social activity (Table 1). For three of the four samples collected, the dolphin group was resting and one group was socializing.

DNA extraction was attempted from all four blow samples. In all cases, DNA concentrations were too low to be read on the Qubit Fluorimeter and no DNA was visible when run on agarose gels, though the extraction positive control (human buccal swab) produced visible high molecular weight DNA and DNA concentration was 5.76 ng/μL. PCR amplification of the mitochondrial D-Loop was unsuccessful for all blow samples under all tested reaction conditions, that is, did not result in any visible bands when run on

Table 1. Summary of drone sampling effort divided by dolphin groups.

Date	Species	Sighting no.	Group size	Total flight duration (h:min:s)	No. of flights	Blow samples collected	Dolphin group activity (number of flights in brackets)
4 May 2018	Humpback	1	2	0:11:30	1	0	Forage (1)
5 May 2018	Humpback	2	2	0:12:00	1	0	Unknown (1)
5 May 2018	Humpback	3	4	0:29:36	4	0	Travel (1) Rest (3)
6 May 2018	Humpback	4	5	0:08:53	2	0	Rest/traveling (1) Socialize (1)
6 May 2018	Humpback	5	9	0:29:46	5	1	Socialize (5)†
7 May 2018	Bottlenose	6	10	0:42:11	16	3	Rest (6)† Socialize (5) Travel (4) Unknown (1)
7 May 2018	Bottlenose	7	12	0:28:41	11	0	Socialize (10) Unknown (1)

† Highlights the dolphin group activity when samples were successfully collected.

agarose gel, but was successful for the positive DNA control. Unfortunately, no DNA was detected in any of the four blow samples collected, despite the amplification attempts in the laboratory.

Discussion

To our knowledge, this study is the first published attempt of using a drone to collect blow samples from free-ranging dolphins. While we were successful in obtaining blow samples, we were unable to extract any genetic information. There are several possible reasons why no dolphin DNA was detected. First, we used the samples of a single blow at a time; second, the drone was 0.5–1 m above the dolphin during sample collection, a difficult altitude to maintain even for an experienced pilot; and third, dolphins often begin to exhale, while their blowhole is still under water making it feasible that we mostly collected seawater. In contrast, in Frère et al. (2010), where dolphin blow DNA was successfully amplified, a sample consisted of four to six blows and was collected just above the blowhole of a captive dolphin holding its head above the water surface. Hence, a lower DNA concentration would be expected in the drone sampling compared to Frère et al. (2010), but the absence of any PCR product means that our protocol requires more work.

Flying the drone <1 m above the surface of the ocean is difficult, and the risk of hitting the water is very high, particularly in choppy sea conditions. The drone itself could be improved with the installation of a collision-avoidance capability that automatically maintains a predetermined altitude from the water surface, reducing workload for the pilot and allowing safer flight at extremely low altitudes. LIDAR, sonar, and optical flow image processing are suitable technologies to explore, but all would incur greater weight, complexity, and cost to the drone. The additional hardware would need to be miniaturized, waterproofed, and integrated to create a flight control system capable of detecting and avoiding wave crests at speed, presenting significant technical challenges to overcome.

The drone pilot needs to see the dolphin blow through the drone's camera feed in order to fly toward it and take a sample. Relative to larger cetaceans, dolphin blow is generally close to the water surface (<1 m), is more transparent, and disperses almost immediately, unlike whale blow that tends to hang in the air for a few seconds. These factors make it harder for the drone pilot, firstly to see the blow from the drone camera and secondly to reach the blow in time to take a sample, before it disperses. We used a video feed of 600 television lines equivalent to 720 × 480 pixels on a seven-inch monitor, but upgrading the live video camera and downlink on the drone to a higher resolution version, and also a larger monitor on the pilot's handset, would increase his/her ability to see the blow and therefore collect samples.

Given the close proximity of the sample material to the surface of the ocean and the difficulties

this presents to multirotor drones, it may be valuable to consider alternative types of remotely operated vehicles (drones), which may be more suited to the task. Remote-controlled boats, ground-effect vehicles, or hovercraft ride on the surface of the water, cannot crash and would hold a petri dish at the right height but would likely present more visual and noise disturbance to the animals and may increase the risk of a collision.

Another challenge of using drones for population genetic studies of dolphins is differentiating individual dolphins and linking the blow sample and subsequent DNA to the correct individual. To minimize our disturbance to dolphins, we attempted to maintain a distance of >100 m between the boat where the drone was launched and the dolphins. Occasionally, the dolphins approached the boat within 100 m. Such large distances may be prohibitive to correct photo-identification of individual dolphins being sampled. This may be overcome with high-definition images captured of the marks on the dorsal fin (used to differentiate between individual dolphins) from the GoPro video on the drone and supplemented by high-quality photo-identification images from the boat if dolphins approach close enough. If dolphins are close to each other, it may prove difficult to sample individuals as multiple dolphins may be sampled simultaneously, which would confound the resulting genetic information. Nonetheless, based on our pilot study and the low volume of material sampled in this study, we would recommend post hoc identification of individual dolphins and collecting multiple samples.

Our DNA extraction method should have been suitable for the small amounts of starting material expected from blow samples. The QIAamp DNA Investigator Kit is routinely used in forensic investigations and performs favorably with other common methods (e.g., Chelex 100; Bogas et al. 2011, Brownlow et al. 2012, Phillips et al. 2012, Ip et al. 2015). The kit has been validated for DNA extraction from as little as 0.1 μL of saliva or 0.01 μL of blood (Qiagen 2015). Frère et al. (2010) used the Qiagen DNeasy Blood and Tissue Kit with success, and others have used the PowerBiofilm DNA Isolation Kit (Apprill et al. 2017), Quick-DNA (Pirotta et al. 2017); in all cases, a greater volume of starting material would have been available, for example, a greater volume of blow obtained from captive dolphins (Frère et al. 2010) or from larger animals (Pirotta et al. 2017). While we anticipate our DNA extraction methodology was suitable to detect dolphin DNA if present in the sample, some improvement to the collection method could be made to enhance the capture of detectable DNA. For example, Frère et al. (2010) have reported using absorbent filter papers in the petri dish to capture DNA in a pilot study; however, this subsequently inhibited DNA extraction and PCR amplification. Whatman FTA cards (GE Healthcare, Chicago, Illinois, USA) that are chemically treated to fix and store nucleic acids at room temperature may be an appropriate alternative to enhance DNA capture and subsequent extraction from blow samples as they have been successfully used for DNA extraction from noninvasive samples of other wildlife (Lucentini et al. 2006). Using a material such as nylon stocking has also been successful in absorbing cetacean blow and may be worth trialing (Hogg et al. 2009).

This trial has provided a useful insight into the approachability of free-ranging dolphins to a drone and their response to it. The success of obtaining samples was dependent on the individual dolphin's activity. When dolphins were socializing, they were not predictable in their surfacing and direction. This made it very difficult to position the drone appropriately so that it would be at a low enough altitude above a dolphin to capture blow. The ideal circumstance to capture blow was to track a dolphin from behind and then swoop low as the dolphin surfaced within range of the blow. Nevertheless, the dolphins apparently perceived the drone several times before diving. In one group of socializing bottlenose dolphins, we observed a tail slap (typically an aggressive behavior). As we could not determine whether it was directed at another dolphin in the group or the drone, following the group was immediately terminated and the drone returned to the boat. No attempt to resample this group was made. On a separate occasion, an individual in a group of humpback dolphins was observed to tail slap twice in conjunction with other surface-active behaviors, while the drone was <5 m from the individual and group. However, these behaviors were observed before and after the drone

sampling attempts and were deemed directed toward conspecifics and not toward the drone. However, at other times during the trial, dolphins appeared to avoid the drone by either changing direction or diving. Consideration should be given to the disturbance potential of the drones and the energetic cost this may have on the targeted dolphins (Fettermann et al. 2019).

When dolphins are socializing or foraging, the directional changes are too frequent and behaviors too unpredictable. Additionally, behaviors such as tail slapping and leaping are more frequent when dolphins are socializing with one another and it is difficult to discern whether these behaviors are in response to the presence of the drone or other dolphins. Furthermore, these unpredictable surface-active behaviors, when the drone is at low altitude (<1 m above the water surface), could potentially result in an injury if contact occurred between the dolphin and the drone. We would recommend attempting to sample the blow of dolphins when they are engaged in an activity such as traveling or resting. This is because the dolphins' movements are generally more predictable during those activities allowing the drone pilot to track the dolphins and anticipate the best time to sample. Nevertheless, the duration of sampling should be limited to ensure disturbance is minimized and short-term and does not have longer term energetic consequences (Williams et al. 2006).

We had originally set out to explore the possibility of an alternative to the traditional PAXARMS biopsy sampling system to collect DNA data (Krützen et al. 2002). A recent study sampling *Sousa chinensis* (similar to *S. sahulensis* targeted in this study) reported an 18% sampling success rate using the PAXARMS biopsy sampling system (Liu et al. 2019) much lower than the 75.8% success rate reported by Krützen et al. (2002) when sampling *Tursiops* sp. While using drones to collect blow samples provides a potentially less invasive sampling alternative to traditional biopsy approaches, we are now of the view that the drone method will not necessarily replace but instead complement biopsy sampling. Each method has its limitations, and these do differ. Biopsy sampling is more invasive and is difficult to employ with dolphins that are wary of vessels and difficult to approach. Sampling by drone does not require close approach by the vessel and is effective where the animals' movement is relatively predictable at the surface. Factors limiting the application of drones in sampling dolphin blow are dolphin speed and maneuverability and potentially their perception of the drone itself at low altitudes. For example, when we encountered socializing groups of humpback dolphins in the Dampier Archipelago, they were relatively easy to approach and biopsy. As detailed above, socializing dolphins are not conducive to drone sampling and, in this situation, biopsy was the best tool to sample dolphin DNA. Therefore, adopting a technique may be circumstantial and discretionary depending on the dolphin activity and approachability. Poor water quality prevents biopsy sampling of humpback dolphins in some locations, for example, Taiwan, because the resulting open wounds could compromise the health of the individuals being sampled (Wang et al. 2008) and, additionally, health assessments are not possible as samples of viruses and microbiome cannot be obtained through biopsy. In both of these circumstances, drones enable the collection of the appropriate samples (Geoghegan et al. 2018) and could potentially benefit particularly small populations in contaminated waters, which are already vulnerable to stressors. Nonetheless, research efforts should be mindful of minimizing the disturbance on dolphins in any attempts to obtain samples (Hodgson and Koh 2016, Ramos et al. 2018).

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Supporting Information

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